



Bioefficacy of Lemongrass and Tea Tree Essential Oils Against House Fly, *Musca domestica*

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Abstract The management of house fly, *Musca domestica* has become immensely important to prevent epidemics of many detrimental diseases. In view of this objective, the present study demonstrates the efficacy of lemongrass (LG) and tea tree essential oils (TTEOs) against *M. domestica*. The TTEO proved to be more lethal against larvae and adults of *M. domestica* depicting an LC₅₀ at 14.88 mg/dm³ which was 17.19 mg/dm³ for LGEO. In contrast, pupicidal

effect of LGEO was much higher (LD₅₀, 14.49 µl/0.25L) as compared to TTEO. The LGEO drastically reduced the total body sugar, glycogen and protein contents by 3.29, 2.95 and 7.56 fold, respectively, contrasting with high influence of TTEO on lipid content of the late 3rd instar larvae. A considerable reduction in gut enzymes secretion was observed due to treatment of EOs thereby altering gut physiology of the insect. Moreover, significant inhibition of acetylcholine esterase (AChE) was also observed with LGEO at LC₅₀ concentration (5.33 mg/ml) inhibiting insect neurotransmission. The gas chromatography-mass spectrometric (GC-MS) analysis of the LGEO showed 12 major compounds dominated by Citral whereas TTEO contained only 5 major compounds. Further analysis by field emission scanning electron microscopy (FESEM) revealed distortion and shrinkage of larval bodies caused by the treatment of EOs. These overall observations brand LG and TT-EOs as potential organic-insecticides against *M. domestica*.

Significance statement The housefly, *Musca domestica* is a menace for the modern civilization due to its role in transmission of fatal diseases. The present manuscript highlights lemongrass and tea tree essential oils as potential biopesticides against *M. domestica*.

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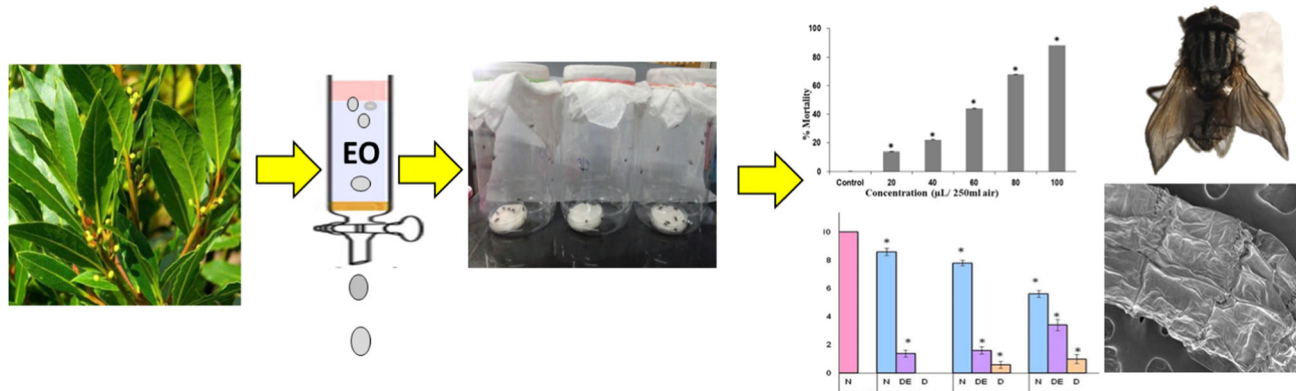
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Graphic Abstract



Keywords *Musca domestica* · Essential oils · Botanicals · Vector-borne diseases · Mortality · GC-MS analysis

Introduction

The holometabolous insect, *Musca domestica* also called house fly plays its part as a vector of many fatal diseases. It has become a worldwide problem for transmission of pathogens such as protozoan cysts, helminth parasites, enteropathogenic bacteria, Turkey coronavirus, enterovirus as well as avian flu [1]. It causes heavy damage to livestock, poultry and humans. It has been very instrumental for the epidemics of dysentery, diphtheria, typhoid, leprosy in humans, whereas fowl cholera and anthrax diseases of poultry and livestock [2]. The frequent movement of flies between animals and rotten food makes them ideal porters for disease causing pathogens. Therefore, the population control of this pest below threshold levels becomes immensely important from the public health viewpoint and has attracted attention of the scientific community worldwide. During the last century chemical control was the only potential option for managing this insect, but in the present era of advanced technologies many prospective alternatives, such as cultural tactics and biological agents, are routinely used for controlling this pest. Moreover, house flies are reported to show resistance to chemical agents such as organochlorines, organophosphates, pyrethroids, etc. [3]. Furthermore, synthetic pesticides are also well-known to harm non-target organisms, cause bioaccumulation in trophic levels, ecological imbalances and environmental pollution. Thus, there is a dire need to prospect for new, promising and sustainable biological agents that could be potential tools for managing the housefly populations

below threshold levels. Prospectively, there are renewed efforts to develop plant-based formulations which could be considered as highly effective, eco-friendly, cheap, biodegradable and safe for humankind as well as the environment. According to Isman, favorable eco-toxicological properties of botanical pesticides make them potentially suitable for use in integrated pest management (IPM) programs [4]. Consequently, essential oils (EO) extracted from plants are reviewed as potential plant-based organic-agents due to their inherent valuable properties.

Since EOs are mixtures of organic compounds that can accomplish multiple functions such as protein denaturation, enzymatic inhibition and membrane disintegration in animals [5]. Some compounds may act as insect growth regulators (IGR) affecting the neuromuscular system of pests and mimic the symptoms of organophosphates. While others absorb through the cuticle and function as fumigants causing respiratory damage [6]. Since every pesticide has its pros and cons but the specialty of plant-based formulations being species-specific and biodegradable makes them an ideal choice for future pest management practices. The EOs from LG and TT are known to be stress relievers bearing many medicinal properties for humans. However, their valorization for pest management in general and house flies in particular has remained to be explored. Thus, in the present study, we investigated the effects of LG and TTEOs against the menacing house fly, *M. domestica*. The efficacy of EOs were tested on different life stages of the model insect by using various biochemical techniques while the morphological alterations imposed by EO treatment were also determined using FESEM. Moreover, attempts were made to explore the composition of EOs under consideration in order to confirm the active ingredients responsible for insecticidal properties.

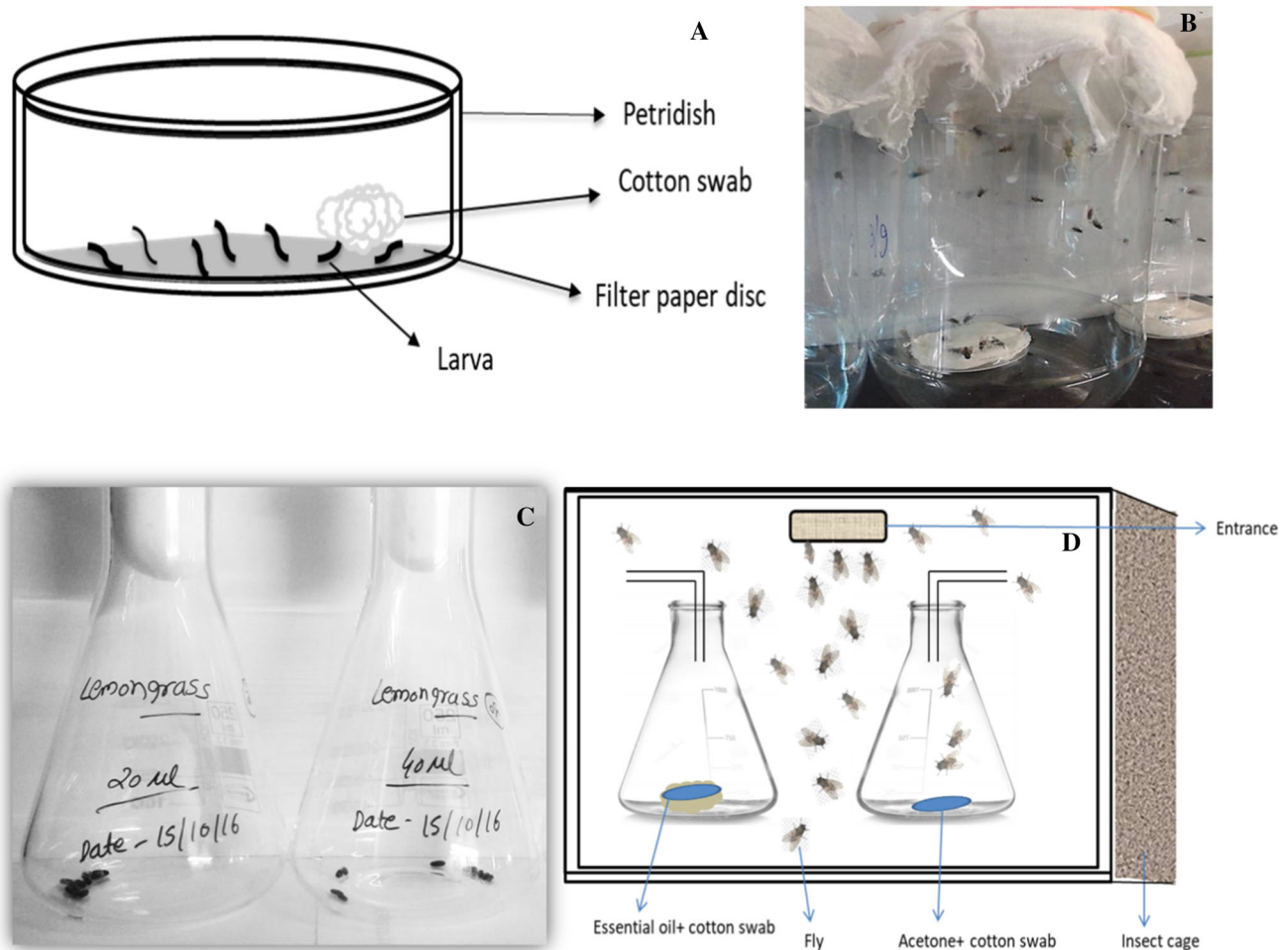


Fig. 1 An outline of the different bioassays carried out in the present study. **a** set up for larvicidal assay, **b** Adultericidal assay carried out in plastic jars containing petri dish sprayed with milk solution as food, **c** Pupicidal assay and **d** Attractant/repellent assay

Material and Methods

Chemicals and Reagents

The EOs of lemongrass (*Cymbopogon citratus*) and tea tree (*Melaleuca alternifolia*) used in the study were procured from Sigma Aldrich, (Millipore Sigma, USA). The EOs were diluted in acetone solution (Merck, corporate, USA) to prepare the doses for different assays or experiments. All other reagents and chemicals used in the study were of analytical or molecular grade unless otherwise mentioned.

Insect Rearing

The culture of housefly, *M. domestica* procured from the national chemical laboratory (NCL), Pune (M.S.), India, was maintained at 28 ± 2 °C and 60–70% R.H. for several generations in plastic jars of 35×15 cm dimension as described previously [7]. The jars were covered with muslin cloth to prevent the flies from escape. The adult

flies were fed *ad libitum* on 10% milk solution (w/v) offered through a cotton swab which served dual purpose for food as well as substratum for oviposition. The food was changed regularly to prevent the contamination by microorganisms. The rod-shaped eggs laid in batches were whitish in color and approximately 1.2 mm in size (Fig. 1a). The eggs after oviposition were stored in different sets of jars for hatching. The newly hatched larvae, creamy whitish in color (Fig. 1b) were fed on milk solution. The first to third larval instars were identified on the basis of size of the larva and time after hatching by following the descriptions of Hussein and Capinera [8]. The collected pupae were kept separate for emergence into adult. Finally, the freshly emerged adults were also used for various bioassays.

Larvicidal Assay

The larvicidal assays were carried out by using the residual film method [7] with slight modifications. Briefly, the

effect of different concentrations of tested oils, were checked on 3rd instar larvae ($n = 10$) to determine the LC_{50} and LC_{90} concentrations. To achieve this, 1 mL of the acetone solution containing desired concentration of EO, were spread uniformly on filter paper disc laid inside the glass petri dish of 90-mm-diameter (Fig. 1a). The petri dishes were left open till solvent evaporation followed by inoculation of larvae ($n \geq 10$). These tests were carried out under strictly maintained rearing conditions for 24 h. However, in control experiments only solvent, i.e., acetone in place of EO was sprayed following a similar procedure to that in test sets. The larvae were fed on cotton swab soaked in milk solution. The actual dose of EO present in 1 mL mixture was calculated by using the following formula considering the area of petri dish as 63.58 cm^2 .

$$\text{Dose/cm}^2 = \frac{\text{concentration present in 1 mL solution}}{\text{area of petri dish}}$$

The larval mortality was determined after 24 h of exposure with EO to calculate the mean mortality for LC_{50} , and LC_{90} values using probit analysis [9]. In case of LGEO, the assays were repeated 7 times while the test with TT was repeated 10 times to get the fair results for larval mortality caused by the tested compounds.

Adulticidal Bioassay

The adulticidal assay on *M. domestica* was carried out according to the method described previously [7] with minor changes. The bioassays were designed such that the flies would have a maximum probability of exposure to the tested concentration of EOs. The adult houseflies ($n = 10$) were transferred to a plastic jar of 1.2 dm^3 having a cotton yarn attached internally to the cap (Fig. 1b). The cotton yarn was sprayed with different concentrations of EO in the range of $10\text{--}100 \text{ mg/dm}^3$ which was dissolved in $10 \mu\text{L}$ acetone. To avoid direct contact of flies with EO, the cotton yarn was covered with a thin muslin cloth. In case of control set, the cotton yarn enclosed in muslin cloth contained only $10 \mu\text{L}$ of acetone. The experiments were replicated 10 times at $28 \pm 2 \text{ }^\circ\text{C}$ for 30 min to calculate the mortality rate caused by EO treatment.

Attractant/Repellent Bioassay

The attractant/repellent assays were carried out by double choice method [7] where 20 individuals of newly emerged adult flies were released in a cage containing 2 conical flasks. One flask contained 0.1% test oil in 5 mL (v/v) of milk, while the other contained acetone as solvent with 5 mL of milk to serve as a control. The EO containing conical flask was fitted with a funnel (10 cm diameter)

outwards to avoid the escape of the flies (Fig. 1d). The number of flies attracted toward test oils, and solvent only in control flask were recorded after 24 h of treatment, and the percent (%) repellency was determined by using the formula;

$$\% \text{ Repellency} = [100(C-T)/C]$$

where C is the number of flies trapped in control flask, T denotes the number of flies trapped in treated flask.

Pupicidal Bioassays

To determine the effect of EO on pupal emergence into adults, two types of assays such as volatile and contact toxicity protocols as described by Kumar et al. [10] were followed. Twenty pupae (3 days old) were placed in 250 mL conical flasks (Fig. 1c) containing respective concentrations ranging from $20 \mu\text{L}$ to $100 \mu\text{L}/0.25 \text{ L}$ of air. The observations were recorded up to 6 days post exposure at $28 \pm 2 \text{ }^\circ\text{C}$ and RH $65 \pm 5\%$, and the percent (%) reduction of emergence into adults or inhibition rate (%IR) was calculated by following the formula;

$$\% \text{ Inhibition rate} = \frac{C_n - T_n}{C_n} \times 100$$

where C_n is number of emerged insects in control set, and T_n corresponds to the number of emerged adults after EO treatment.

Effect of EOs on Sugar, Glycogen and Lipid Contents

To determine the effect caused by EO treatment on the larval body, 3rd instar larvae ($n = 10$) were exposed to LC_{50} concentrations of test oils for 24 h. After treatment, the larvae were sacrificed to estimate the biochemical components like total sugar, glycogen and lipid contents. The body contents were homogenized in 0.2 mL Sodium sulfate solution (w/v) followed by addition of 0.8 mL methanol in chloroform (1:1, v/v). The homogenized mixture was centrifuged at 3000 rpm for 2 min, and the supernatant was harvested for glycogen estimation by Anthrone method [11]. The pellet obtained was further suspended in 3 mL of dH_2O and centrifuged at 3000 rpm for 2 min to observe the formation of 2 layers. The upper aqueous layer was used for sugar analysis by the method of Van Handel and Day [11] while the bottom layer chiefly formed of chloroform containing lipids was mixed with 0.2 mL H_2SO_4 then heated for 10 min at $90 \text{ }^\circ\text{C}$. The color change caused by the addition of 5 mL Vanillin reagent was measured at 625 nm spectrophotometrically (Cary 60, Agilent technologies, USA). For sugar and glycogen content, glucose was used as standard whereas Soybean oil was used in case of lipids. The total body proteins were

determined by Bradford assay [12] using BSA as standard after homogenization of the larval body in 1 mL of lysis buffer (0.1 M PMSF, 0.1 M tris Cl, 1 M EDTA, 1 M NaCl, 1 M DTT, 20% glycerol) followed by centrifugation at 5000 rpm for 5 min. The biochemical components under consideration were determined in terms of micrograms (μg)/larva, derived from the standards. These tests were repeated at least 5 times to get the constant results.

Effect of Essential Oil on Gut Enzymes

To check the effect of EO treatment on gut enzymes, gut regions of control and treated larvae were homogenized in PBS (pH 7.4) then centrifuged as described above in protein assay. The supernatant obtained was subjected to different enzyme assays such as α -amylase, α - as well as β -glucosidase activities. The α -amylase activity was carried out by dinitrosalicylic acid (DNSA) procedure [13]. Briefly, 20 μL of the enzyme extract, in 50 μL of universal buffer (pH 7.4) was mixed with 25 μL of soluble starch (1%, w/v). The reaction mixture was incubated at 35 $^{\circ}\text{C}$ for 30 min followed by the addition of 85 μL DNSA reagent and heated in a boiling water bath for 10 min to terminate the reaction. After cooling the reaction mixtures on ice, absorbances were measured spectrophotometrically at 540 nm.

The α - and β -glucosidase activities were determined as per the method described previously [7] using *p*-nitrophenol as standard. The reaction mixture contained 50 μL of enzyme sample with 75 μL of 5 mM *p*-N- α -D-glucopyranoside or *p*-nitrophenyl- β -D-glucopyranoside (as substrates) for α - and β glucosidase, respectively. To the reaction mixture, 125 μL of universal buffer (100 mM, pH-7.0) was added, followed by incubation for 30 min at 25 $^{\circ}\text{C}$. Reactions were stopped by 2 mL of 0.1 M Sodium carbonate or NaOH, and absorbances were read spectrophotometrically at 405 nm. The enzyme activity is defined as the amount of enzyme required to liberate 1 mg maltose under standard assay conditions.

Acetylcholinesterase (AChE) Activity

Acetylcholinesterase (AChE) activity was assayed by using Ellman's reagent, DTNB [14] at 412 nm. The reaction mixture composed of 10 μL enzyme sample with 180 μL Ellman's reagent was diluted with 10 μL dH₂O in the presence of 20 μL Acetylthiocholine (20 mM) as substrate. The enzyme activity was measured in U/mg of extract.

Gas Chromatography- Mass Spectrometric (GC-MS) Analysis of EOs

To check the chemical composition of the tested EOs, we employed GC-MS approach using GC-MS-TQ8030 apparatus, equipped with RTX 5MS column at an ionizing voltage of 70 eV. The mass spectra obtained were compared with the relevant compounds in NIST11 library of GC-MS data system as well as already reported literature. The GC-MS of Lemongrass EO analysis was carried out by the method of Mirghani and coworkers [15]. The GC consisted of primary heating from 50 to 150 $^{\circ}\text{C}$ at 6 $^{\circ}\text{C}/\text{min}$ followed by an increment from 10 to 250 $^{\circ}\text{C}$ which was maintained for 10 min. Injector temperature was maintained at 220 $^{\circ}\text{C}$, and the MS transfer line temperature was set at 290 $^{\circ}\text{C}$. Helium was used as a carrier gas at a flow rate of 1 mL/min and a split ratio of 1:30. However, for GC-MS analysis of TTEO, slight modifications were made by changing the column temperature from 60 to 240 $^{\circ}\text{C}$ with an increase of 3 $^{\circ}\text{C}/\text{min}$, maintained for 35 min.

Field Emission Scanning Electron Microscopic (FESEM) Analysis of Treated Larvae

To study the effect of EOs on insect morphology, 10 larvae were exposed to LC₅₀ concentrations for 24 h. After treatment, the larvae were prepared for FESEM analysis to observe the changes in integument and surface structures. The morphology of the treated and control larvae were observed in an electronic microscope (FESEM 450, FEI Nova Nano, USA) complemented with EDS system at a voltage of 3 kV. For FESEM visualization, the samples were primarily fixed in 2.5% glutaraldehyde for 12 h, then washed with distilled water for 20 min followed by a secondary fixation with 4% Osmium tetroxide. The samples were further dehydrated with different ethanolic grades (25, 50, 75, 90 and 100% ethanol) each for 10 min at RT. Finally, the samples were subjected to critical point drying (CPD) and mounted on carbon tape followed by sputter coating with 100 \AA gold.

Statistical Analysis

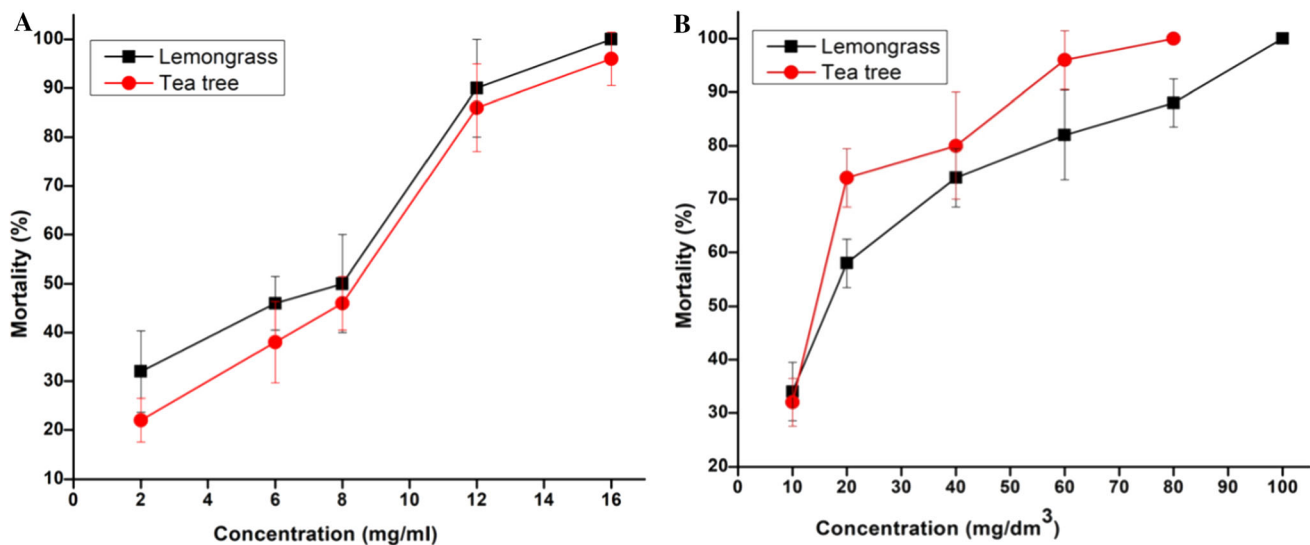
All the bioassays were independently replicated five or more times unless mentioned. The data obtained was statistically analyzed in SPSS software version 22 (IBM SPSS, NY, USA), and the obtained results are reported as mean \pm standard error mean (SEM) of five or more replicates. One-way analysis of variance (ANOVA) was carried out by using Tukey's test ($p < 0.001$). The LC₅₀ and LC₉₀ values with their 95% confidence limits were

Table 1 Larvicidal and adulticidal activities of tested essential oils against *M. domestica* after 24 h of treatment depicting LC₅₀ and LC₉₀ concentrations

Essential Oil	Insect stage	LC ₅₀ (mg/ml) [#] (mg/dm ³) ^{##}	95%Confidential limit (LC ₅₀)		Regression Equation	LC ₉₀ (mg/ml) [#] (mg/dm ³) ^{##}	Chi square
			LCL	UCL			
Tea tree	Larvae	4.94	3.98	5.84	$y = 0.31 - 1.53x$	9.08	17.00*(5)
	Adult	14.88	6.65	25.01	$y = 0.41 - 608x$	46.27	16.67*(5)
Lemongrass	Larvae	5.33	2.74	7.41	$y = 0.209 - 1.114x$	11.46	41.31*(5)
	Adult	19.17	3.41	31.69	$y = 0.24 - 0.465x$	72.05	11.87*(5)

LC₅₀ and LC₉₀ are lethal concentration at which 50% and 90% population died, respectively. # Unit representing the larval mortality; ## unit used for adulticidal activity

*Significant at $p < 0.001$. Each value represents mean of five replicates

**Fig. 2** Larvicidal (a) and adulticidal (b) activities of tested essential oils on *M. domestica* larvae. Each value represents mean of five or more replicates

determined by probit analysis, which helped to analyze the dose–mortality response [9].

Results and Discussion

Larvicidal Activity

Among the EOs tested against the larvae of *M. domestica*, it was observed that TTEO proved to be more effective as compared to LGEO as depicted by the lower concentration required for 50% mortality of the larvae (Table 1). The TTEO showed higher larvicidal activity at LC₅₀ 4.94 mg/mL (actual dose was 0.0776 $\mu\text{g}/\text{cm}^2$) which was very low and statistically significant ($p < 0.001$ level) than LG essential oil requiring 5.33 mg/mL (actual dose was 0.0838 $\mu\text{g}/\text{cm}^2$) to kill 50% of pest populations. Though it is not surprising to observe the increased mortality rate with increased concentrations of EO but the time required

by TTEO to achieve LC₅₀ which was only 24 h is quite fascinating (Fig. 2a). It can further elucidate that short-term exposure of larvae to lethal doses can markedly increase their mortality over time, and thus reduce the population levels below threshold. Our observations were in line with Shalaby and coworkers who reported that lemon oil shows higher larvicidal activity [16]. Similarly Bosly, [17] stated that EOs from *Mentha piperita* and *Lavandula angustifolia* caused mortality and prolonged larval stages in *M. domestica* thereby show a potential insecticidal effect.

Adulticidal Assay

Since both the EOs exhibited adulticidal activities but the activity depicted by TT compound was significant with LC₅₀ and LC₉₀ concentrations of 14.88 mg/dm³ and 46.27 mg/dm³, respectively (Table 1). However, LGEO showed LC₅₀ at 19.17 mg/dm³ and LC₉₀ with 72.05 mg/

Table 2 Attraction/ repellency potential of essential oils toward *M. domestica*

Sr. no.	Essential oil	Mean no. of flies attracted in control	Mean no. of flies attracted in test	% Repellency
1	Lemongrass	8.2 ± 0.37	1.6 ± 0.4	67.34
2	Tea tree	10.8 ± 0.58	1.4 ± 0.24	77.04

dm³, showing 100% mortality at concentration 100 mg/dm³ which was higher than TTEO (80 mg/dm³) (Fig. 2b). The Chi square values and 95% confidence limits indicated that the results obtained were statistically significant at $p < 0.001$. A similar trend like larvicidal activity was observed in case of adulticidal activity; with increased dose the percent (%) mortality also increased showing positive correlation with the bioefficacy of the tested compounds. Similar inferences have been reported by Shalaby and coworkers [16] while testing the effect of peel oil from grapefruit on *M. domestica*.

Attractant/Repellent Bioassay

In this assay, TTEO showed 77.04% repellency which was slightly higher than the repellency potential of LG, (i.e., 67.34% repellency) at a concentration of 0.1% (Table 2). Recently, the repellent activity of *Cymbopogon citratus* (lemongrass) EO against mosquitoes has been examined by Oyedele et al. [18]. Jayasinghe and Fujimoto, [19] stated that repellent potential to *C. citratus* is majorly imparted by its dominant compound geraniol while testing them against *Sitotroga cerealella* and *Tribolium castaneum*. However, Kumar et al. [10] have revealed that some monoterpene aldehydes such as Linalool, linalyl acetate, Citral a and

Citral b are the main factors responsible for repellent activity of *C. citratus*.

Pupicidal Bioassay

The literature related to deformity of insects by EO is scanty, and the underlying molecular mechanism remains unclear. The pupicidal assay carried out by two different methods proved that contact toxicity also known as topical method is more effective than fumigation approach for housefly control with LG and TTEOs. In fumigation assay, the lethal dose evaluated by probit analysis showed significant activity with LGEO which exhibited LD₅₀ and LD₉₀ values at 14.49 µL/0.25L and 37.45 µL/0.25L, respectively. The TTEO showed LD₅₀ and LD₉₀ concentrations as 29.68 µL/0.25L and 59.51 µL/0.25L. In fumigation assay LGEO was found to be more toxic against pupae than TTEO (Fig. 3). In case of contact toxicity method none of the flies emerged into normal adults ensuring either 100% mortality of the pupae or deformities after emergence due to EO treatment. The LGEO showed complete (100%) inhibition of pupae at a concentration of 100 µL/0.25L of air displaying more toxic nature, whereas the concentration of TTEO required for 100% inhibition was found to be 80 µL/0.25L of air displaying more toxic nature. With increased doses in fumigation assay the inhibition rate (IR) also increased in both the oils showing maximum deformities in adults. The higher proficiency (100% inhibition) in the contact toxicity method for pupicidal effect could be attributed to the collapse of the insect neural system, therefore causing instant and complete knockdown of the flies which was also reported by Kumar et al. [10] while testing the effect of crude oils from *E. globulus* and *M. piperita* on *M. domestica*.

Further, it was observed that LGEO is more effective on pupae as compared to TTEO causing adult deformation, shrinkage of wings and even producing pupal-adult intermediates (Fig. 4). However, such effects were also observed by Dimetry et al. [20] while examination of volatile oil from *P. nigra* on 3rd instar larvae of *M. domestica*. Some authors have observed that treatment of *T. vulgaris* and *Z. officinale* EOs on *C. albiceps* produces larval-pupal or pupal-adult intermediates with crumpled wings. The effect of EO from LG and TT are more

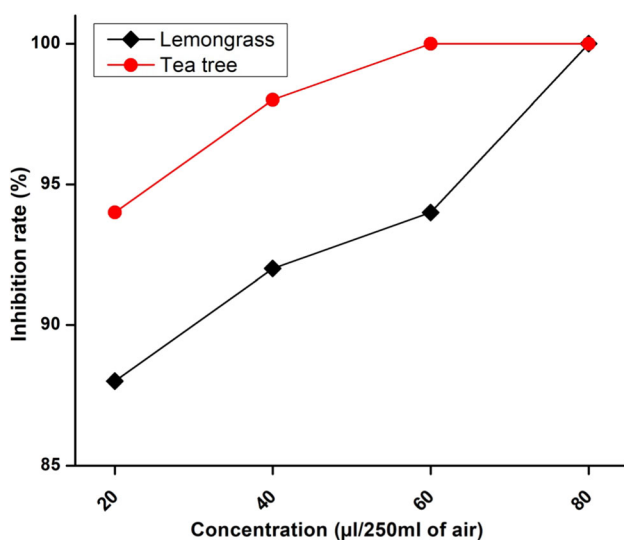


Fig. 3 Percentage inhibition on pupal emergence due to Lemongrass (a), and Tea tree (b) essential oils against *M. domestica*

Fig. 4 Inhibition of pupal emergence into adults (a) caused by the essential oil treatment; (b) shows the deformation of adult undergoing emergence

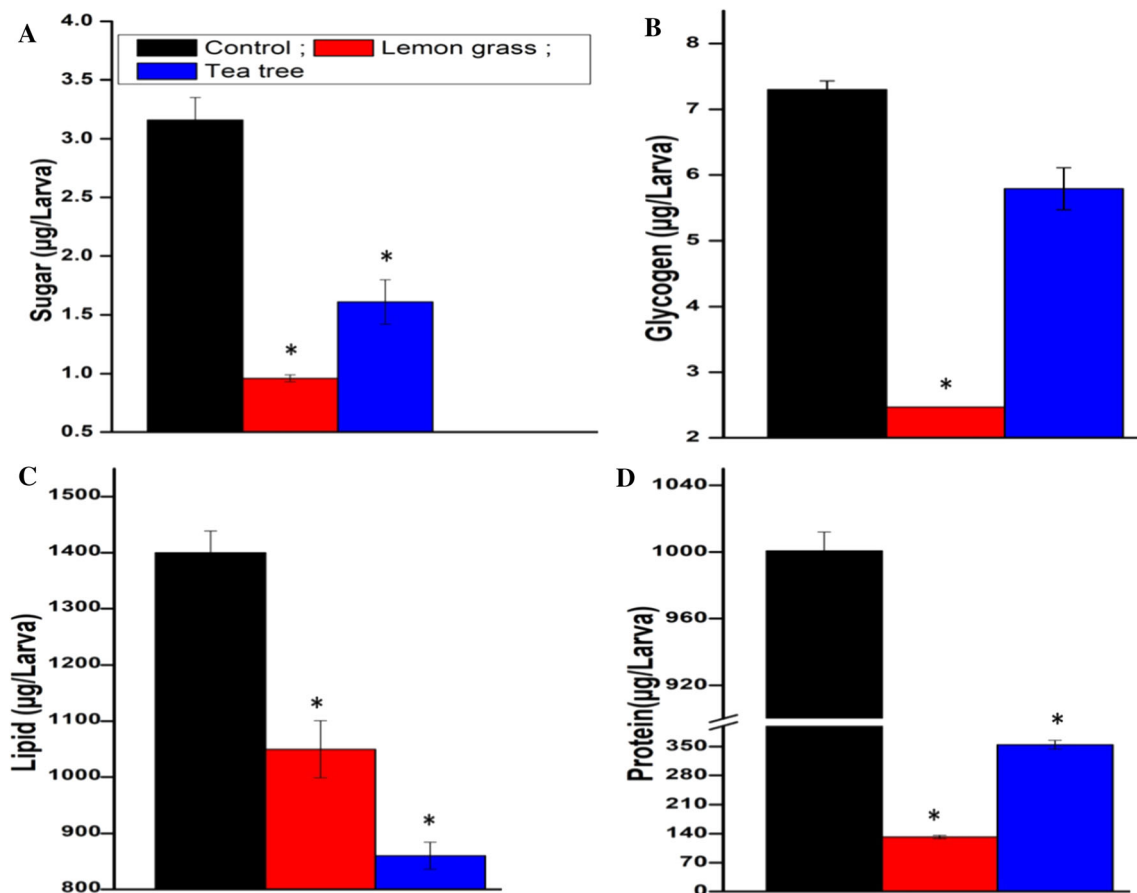
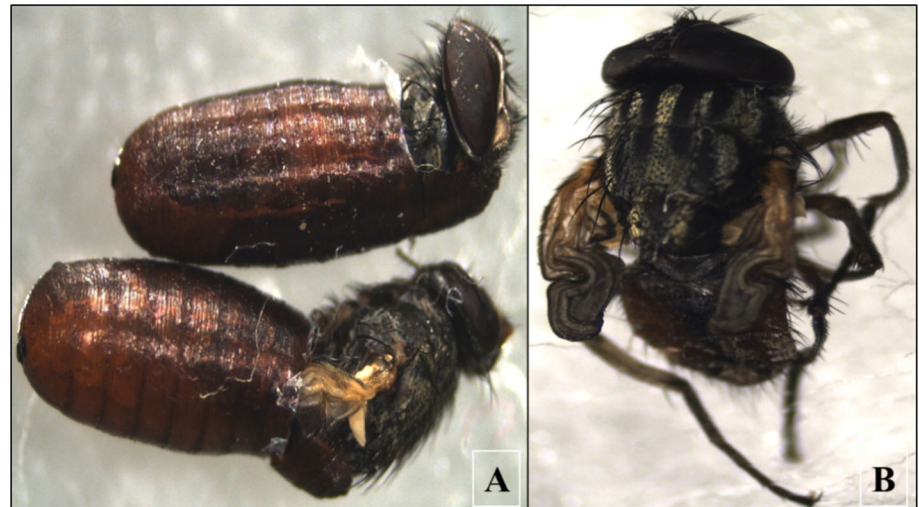


Fig. 5 Effect of EO treatment on the biochemical reserves of house fly, *M. domestica*. A; total body sugar, B; total body glycogen, C; total body lipid content, and D; total body protein. Data analyzed by One-

way ANOVA, followed by Tukey's test and significance at $*p < 0.001$ compared with control

promising than *C. aurantifolia* peels or *Citrus sinensis* which show only 70% fumigant effectiveness against houseflies [21]. The difference in efficacy between contact toxicity and fumigation assays may be due to the different

percentage contributions of chemical components in two phases of oils. Further in the fumigation assay it was observed that % inhibition of house flies is dose dependent because the increased dose of EOs causes increase in % IR

of pupae and increased chances of deformities in adult flies.

Effect of EO on Biochemical Components of Larval Body

Since the nutritional reserves like lipids, sugars and proteins are vital for proper growth and development of the insect we explored the impact of EOs on these metabolites in *M. domestica*. The LC₅₀ concentrations of EOs showed a notable influence on total body sugar, glycogen, lipid and protein content of third instar larvae. Comparably the LGEO exhibited high influence (reduction) over the total sugar, glycogen, and protein contents, whereas TTEO showed a significant effect on total lipid content in *M. domestica* larvae. The LGEO drastically reduced the total sugar, glycogen and protein contents by 3.29, 2.95 and 7.56 fold, respectively, while TTEO reduced the lipid content of larvae by 1.62 fold after treatment at LC₅₀ concentration for 24 h (Fig. 5). Our inferences were consistent with the observations of Senthilkumar and his colleagues, [22] who reported a decrease in the concentration of proteins, lipids and carbohydrates by EOs. However, the reduction of glycogen and sugar content after treatment could be due to their involvement to combat the chemical stress imposed by the tested compounds. Hence, treatment with EOs might have possibly interrupted the metabolic processes and resulted in larval mortality [7]. The reduction of protein content by exposure to plant extracts is frequently reported in the literature. According to Shakoori and Saleem, [23] reduced level of proteins after treatment with EOs may be due to the breakdown of proteins into their amino acids, which help the insect for survival. Similarly, the reduced amount of sugar content in treated larvae of *M. domestica* could have occurred due to over exploitation of carbohydrate resources under stress conditions for proper maintenance of physiological metabolism. However, Khosravi et al. [24] stated that in *G. pyloalis*, sugar content is used to meet energy expenses for detoxification of *A. annua* extract which ultimately leads to exploitation of lipid contents of the body. Our results were in close agreement with the conclusions of Canavoso et al. [25] who detected reduction of lipids in the larvae of *S. littoralis* after treatment with lemon and garlic essential oils. The overall reduction in biochemical contents of the larvae due to EO treatment results in poor metabolism consequently leading to deformities or making the individuals prone to opportunistic diseases by pathogens as compared to normal larvae. Therefore, from the inferences of this test it can be concluded that tested EOs possess remarkable bioefficacy against nuisance causing pest i.e., house fly.

Effect of EOs on Gut Enzymes of *M. domestica*

The gut enzymes are key players for digestive physiology and health of all animals. Since house flies commonly dwell on sugary foods, their digestion mostly depends on amylases and glucosidases. Therefore, we determined the effect of EO on amylase and glucosidase activities in house fly larvae. Since some EOs are known to alter the digestive physiology of the insects, we investigated the effect of tested compounds on house fly larvae. However, we observed a significant difference in amylase activity between control and treated sets. The α -amylase activity was drastically reduced from 0.21 to 0.074 ± 0.001 U/mg protein and 0.1064 ± 0.004 U/mg protein (Fig. 6). Similarly, a considerable reduction of α - and β -glucosidase activities were observed after treatment with EOs. In α -glucosidase activity, LGEO proved to be more effective with a reasonable reduction from 2.45 U/mg of protein to 1.95 ± 0.03 U/mg of protein followed by TTEO (2.37 ± 0.07 U/mg of protein). However, in β -glucosidase activity, LG showed a higher effect, better than TTEO. The β -glucosidase activity was decreased from 3.06 ± 0.07 U/mg of protein in control larvae to 2.65 ± 0.04 U/mg of protein by LG and 2.75 ± 0.02 U/mg of protein with TTEO. According to Mehrabadi et al. [26], if the action of the amylases is inhibited, it causes energy shortness, and ultimately death of the organism which supports our observations of larvicidal bioassay. The inhibition of α -amylase activity in the treated larvae could be a cytotoxic effect on epithelial cells of the insect midgut which synthesizes it. Further, the authors suggested that treatment

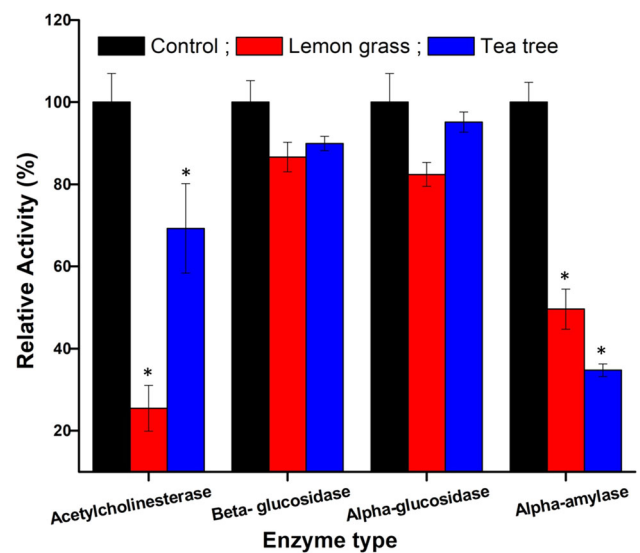


Fig. 6 Effect of essential oil treatment on the activity of gut enzymes and acetylcholinesterase in *M. domestica*. Data analyzed by One-way ANOVA, followed by Tukey's test and significance at $*p < 0.001$ compared with control

with EO causes lysis of cell wall, damages cytoplasm and affects lipid and protein contents [27]. However, in the case of α - and β -glucosidase activities, our finding was consistent with previous reports where *H. rury* larvae treated with sub lethal concentrations of biopesticides showed reduction of α - and β -glucosidase activities.

Acetylcholinesterase (AChE) Activity

The neurotransmitter AChE is pivotal for insects and higher animals. It plays a prominent role in cholinergic synapse in insects. The AChE elucidates the neurogenic toxicity of chemicals on insects, and in this study, it was observed that tested EOs displayed significant effect on acetylcholinesterase activity. Among the compounds, LGEO affected the neurotransmission by the inhibition of AChE activity to a greater extent (0.00288 ± 0.0002 U/mg of Protein) followed by tea tree (0.00785 ± 0.0005 U/mg of Protein) as compared to normal larvae (0.01134 ± 0.0003 U/mg of protein). The inhibition of AChE is largely imparted by the presence of monoterpenes present in aromatic compounds like EOs [27]. These results are in line with our previous observations [7] where EO from *Laurus nobilis* showed higher inhibitory activity of AChE in *M. domestica*. There are several reports concluding that monoterpenoids and many plant volatiles cause insect mortality by inhibiting acetylcholinesterase enzyme [28].

GC-MS Analysis of EOs

Plants serve as a reservoir for many active compounds with distinguished properties which attributes to their commercial value. The EOs extracted from plants with characteristic aroma are composites of oxygenated terpenes, hydrocarbons and sesquiterpenes, which impart special economical significance to these secondary metabolites. Since EOs differ in their chemical composition therefore, characterization becomes an important process to decipher their different chemotypes. The GC-MS is an ideal technique for the qualitative and quantitative determination of volatile and semi-volatile organic compounds in EOs. These components in EO are frequently identified by comparison of their unique retention indices and characteristic mass spectra.

The GC-MS analysis of the LGEO revealed 12 major compounds which show the highest mass spectra ratios (Fig. S1). The major components present in the oil were Citral (37.48%) followed by Geranyl methanoate (26.88%), acetone (10.66%) and cyclobutane (4.58%) (Table S1). In case of TTEO, we observed 5 major compounds such as Terpinen-4-ol (45.78%) > γ -terpinene (16.52%), > 4-carene (8.75%), > β -Cymene (7.98%)

followed by the least proportion of α -Terpineol (4.48%). Lemongrass is generally known to contain citral as a major component which is a complex of bioactive isomers like neral and geranial. Huynh, [29] reported that citral also known as 3, 7-dimethyl-2, 6-octadienal is a natural mixture of isomeric acyclic monoterpene aldehydes, i.e., geranial (transcitral, citral A) and neral (cis-citral, citral B). However, earlier researchers [30] have stated that TTEO contains 3 major constituents such as 4-terpineol, γ -terpinene and α -terpinene, the distribution and abundance of these constituents in EOs depends on many factors such as genetic variations and geographical conditions of the plant species.

Field Emission Scanning Electron Microscopic (FESEM) Analysis

To have a clear visualization of the morphological alterations induced by EO treatment to the house fly larvae, the FESEM analysis evaluated the ultra-structural changes in the larval integument. The marked changes caused by EO treatment are shrinkage of body segments, along with withered anterior segments (Fig. 7). However, in case of control set where larvae were treated with acetone only, the body appearance was clear and smooth without any distorted structure. Like LG, the TTEO was also found to cause shrinkage and distortion of the larval body. Recently, Kumar et al. [10] also reported similar effects on surface morphology of *M. domestica* larvae due to treatment of *E. globules* EO. They further elucidated that *E. globulus* oil causes surface shrinkage at spinose rings with protruding intersegmental grooves. The surface aberration on insect larva could be caused due to absorption of plant extracts to the larval gut or by ingestion through the cuticle and gut epithelial cells. Similarly, Kumar et al. [10] also observed an effect on proliferation of spinose cells after treatment with EO. According to Pérez-Serrano et al. [30] loss of integument organization is a result of stress responses to EO toxicity.

During the last few decades, numerous studies have tested the efficacy of plant-based formulations against mosquitoes which is largely lacking in case of house flies. Taking into consideration, we determined the effect of LG and TTEOs on different forms of *M. domestica*. As evident from the observations, LG and TT oils showed potential bioefficacy against different life stages of the housefly. Since LG and TTEOs significantly reduced the metabolic contents and inhibited the enzymes like AChE, α -amylase, etc. the present study signposts the insecticidal repertoire of tested compounds for the sustainable management of houseflies. The inhibition of pupal emergence into adults together with larvicidal and adulticidal effects also suggest the formulation of these botanicals for potential industrial

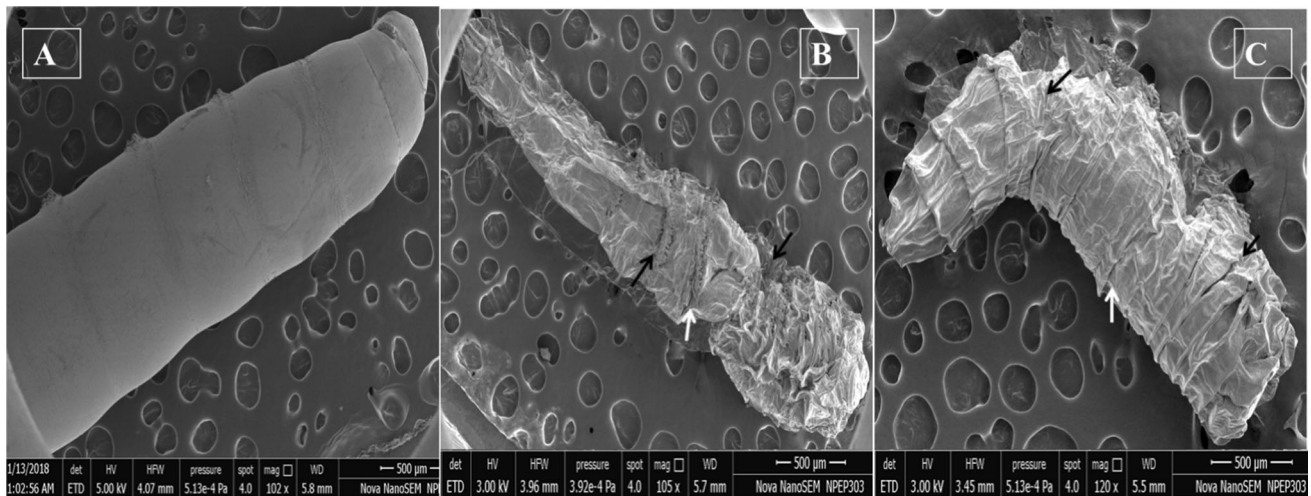


Fig. 7 The ultra-structural view of the alterations cause by essential oil treatment to the surface of housefly larvae for 24 h. Scanning electron microscopic **a**; smooth and normal morphology of the control larvae, **b**; morphometric shrinkage of the larval integument caused by

treatment with lemongrass EO, **c**; morphometric shrinkage and rupturing of body contents caused by treatment with tea tree EO. Arrows indicate the marked changes caused by the treatment of essential oils

applications. The tested oils can thus be easily formulated as microemulsions or nanoemulsions in water or eco-friendly solvent systems. The microemulsions are known to enhance bioefficacy and provide long-term stability to the botanicals. Being volatile, the EOs can also be dissolved in solvents and kept at desired places inside buildings whereby they function as strong repellents for houseflies. In conclusion, LG and TTEOs could prove as important candidates for pest control strategy against houseflies due to their intrinsic larvicidal, pupicidal and adulticidal properties while LG may also act as an effective repellent and ovipositional deterrent for closely related species. However, for field trials and formulation of tested EOs a comprehensive understanding of their mechanism of action on model insect becomes imperative to avert the effects on non-target fauna. Therefore, our future perspective will be to characterize the transcriptome of *M. domestica* in response to EO treatment to better understand the mechanisms associated with their insecticidal activities.

Conclusion

To determine the insecticidal potential of EOs from LG and TT against *M. domestica* different experiments were carried out on different stages of the model insect. In most of the experiments, tested compounds showed significant effects for the control of *M. domestica*. In adulticidal and repellency assays, TTEO proved more effective than LGEO. In contrast, LGEO showed more potential as fumigant for (14.49 μL/0.25 L of air) pupae of house fly. The present study concludes the significant efficacy of EOs

under consideration against *M. domestica* which could be employed to breakdown its population below threshold levels to prevent the menace of some vector-borne diseases. The treatment of TTEO significantly decreased sugar, glycogen and lipid contents whereas LGEO highly affects the total protein content of *M. domestica*. A considerable reduction of α - and β - glucosidase activities was found in treated larvae as compared to the control. The above all findings of the present study brands TT and LGEOs as potential agents for eco-friendly management of houseflies under IPM program encouraging field trials.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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